Vaccination of Colorectal Cancer Patients with Modified Vaccinia Ankara Encoding the Tumor Antigen 5T4 (TroVax) Given Alongside Chemotherapy Induces Potent Immune Responses

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Abstract

Purpose: The attenuated strain of vaccinia virus, modified vaccinia Ankara (MVA) encoding the tumor antigen 5T4 (TroVax), has been evaluated in an open-label phase II study in metastatic colorectal cancer patients. The primary objective was to assess the safety and immunogenicity of TroVax injected before, during, and after treatment with cycles of 5-fluorouracil, folinic acid, and oxaliplatin.

Experimental Design: TroVax was administered to 17 patients with metastatic colorectal cancer. In total, 11 patients were considered to be evaluable for assessment of immunologic responses having received a total of six injections of TroVax, administered before, during, and following completion of chemotherapy. Antibody and cellular responses specific for 5T4 and MVA were monitored throughout the study.

Results: Administration of TroVax alongside 5-fluorouracil, folinic acid, and oxaliplatin was safe and well tolerated with no serious adverse events attributed to TroVax. Ten of the 11 evaluable patients mounted 5T4-specific antibody responses with titers ranging from 10 to >1,000. IFNγ enzyme-linked immunospot responses specific for 5T4 were detected in 10 patients with precursor frequencies exceeding 1 in 1,000 peripheral blood mononuclear cells in 4 patients. Of the 11 evaluable patients, 6 had complete or partial responses. 5T4-specific immune responses, but not MVA-specific immune responses, correlated with clinical benefit.

Conclusions: Potent 5T4-specific cellular and/or antibody responses were induced in all evaluable patients and were still detectable during the period in which chemotherapy was administered. These results suggest that TroVax can be added to chemotherapy regimens without any evidence of enhanced toxicity or reduced immunologic efficacy and may provide additional clinical benefit.

Cancer remains one of the biggest causes of mortality in the developed world. For nonmetastatic cancer, surgical resection offers the best chance of a complete cure. However, when the cancer has metastasized, chemotherapy is the most commonly used treatment but is rarely curative and is often associated with significant toxicity. Active specific immunotherapy represents a promising treatment option, which relies on the induction of an efficacious immune response without accompanying deleterious autoimmune reactions. The identification of a tumor-associated antigen, which is expressed on the tumor target (both primary and metastases) but which is absent on all (or at least most) normal tissues, is important for safe and targeted immunotherapy.

The human oncofetal antigen 5T4 is a 72-kDa membrane glycoprotein that is expressed at high levels on the placenta and also on a wide range of human carcinomas, including colorectal, renal, gastric, and ovarian (1–3). Overexpression of 5T4 is associated with metastatic spread and/or poor prognosis in patients with colorectal (4), gastric (5), and ovarian (6) carcinoma. Transfection of tumor cells with 5T4 cDNA results in increased cell motility, suggesting that expression of this molecule may induce metastatic properties (7, 8). The restricted expression of 5T4 on normal tissues and high prevalence on many common human carcinomas make 5T4 an attractive target for cancer immunotherapy. Furthermore, its surface expression means that it could potentially be a target for both cytotoxic T-cell (CTL) and antibody-mediated effector responses.

For a cancer vaccine to be successful, it must stimulate a potent immune response specific for the target antigen. Several

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tumor-associated antigens have been engineered into vaccinia virus vectors [including modified vaccinia Ankara (MVA)] and the recombinant vaccines shown to induce tumor-associated antigen–specific immune responses in cancer patients (9–11). MVA encoding 5T4 (TroVax) was tested previously in a phase I/II trial in stage IV colorectal cancer patients. This study showed the product to be well tolerated and to induce 5T4-specific immune responses in the majority of patients (12).

Given the encouraging data from the phase I/II study, we wanted to assess the effect of chemotherapy on the ability of TroVax to elicit an immune response. The use of immunotherapy alongside chemotherapy has been viewed as counterintuitive because the latter may suppress the immune system (13). Here, we report on the safety and immunologic efficacy of TroVax administered to colorectal cancer patients alongside 5-fluorouracil (5-FU)/folinic acid and oxaliplatin.

Patients and Methods

Patient characteristics. This phase II trial was an open label study of TroVax administered by i.m. injection to patients with advanced colorectal cancer receiving 5-FU/folinic acid plus oxaliplatin as first-line therapy. All patients had histologically proven colorectal cancer, a WHO performance status of 0, 1, or 2, a life expectancy of ≥3 months, were aged ≥18 years, and had adequate hematologic and liver function. The trial protocol was approved by the United Kingdom Gene Therapy Advisory Committee and the study conducted under a Clinical Trial Exemption granted by the Medicines and Healthcare Products Regulatory Agency (formerly the Medicines Control Agency). The trial was approved by the Local Research Ethics Committees and informed consent was obtained from each patient before enrollment.

Clinical trial design. On entering the trial, each patient underwent chest, abdominal, and pelvic computed tomography (CT) scans to quantify tumor metastases. Further scans were scheduled at weeks 14 and X + 8. Patients received OxMdG (oxaliplatin at 350 mg and the modified de Gramont regimen of calcium folinate at 350 mg simultaneous 2-h i.v. infusion; 5-FU at 400 mg/m^2 i.v. bolus; and 5-FU at 2,400 mg/m^2 i.v. infusion over 46 h) at 2-week intervals starting at week 4 with up to 12 cycles being administered, depending on clinical response and tolerance. Two TroVax immunizations were given before chemotherapy (weeks 0 and 2), two during (weeks 11 and 17), and two following completion of chemotherapy (weeks X + 2 and X + 6; completion of chemotherapy is indicated as week X). Patients received ~5 × 10^8 plaque-forming units TroVax via i.m. injection in a volume of 1 mL into the deltoid muscle. Blood was taken after each immunization to assess the induction of immune responses to 5T4 and MVA (see Fig. 1 for sampling schedule). In addition, the plasma concentration of the surrogate marker, carcinoembryonic antigen (CEA), was measured throughout the trial.

Antigens. Purified recombinant 5T4 protein (14) was used to monitor antibody responses (by ELISA) and cellular responses [by IFNγ enzyme-linked immunospot (ELISPOT)]. In addition, overlapping 5T4 10mer peptides (Mimotopes), CEF peptides (a pool of CTL epitopes derived from CMV, EBV, and Flu, Mabtech), and MVA were used to measure cellular responses.

Measurement of antibody responses. 5T4- and MVA-specific antibody titers were determined by ELISA as described previously (15). Antibody titers were defined as the greatest dilution of plasma at which the mean absorbance of the test plasma was ≥2-fold the mean absorbance of the negative control (normal human plasma) at the same dilution. A positive antibody response due to vaccination was reported if the postinjection titer was ≥ 2-fold the titer determined before TroVax immunization.

Measurement of IFNγ ELISPOT responses. The IFNγ ELISPOT was used to monitor cellular responses throughout the trial. Briefly, frozen peripheral blood mononuclear cells (PBMC) were thawed and incubated in medium overnight at 37°C, 5% CO_2 before use. ELISPOT plates (polyvinylidene difluoride, Millipore) were coated with an anti-IFNγ capture antibody (IFNγ ELISPOT ALD kit, Mabtech). Following blocking, 2 × 10^5 PBMCs were added to each well and incubated overnight at 37°C, 5% CO_2 with the appropriate antigen (depending on PBMC availability, the same panel of antigens was used for all patients and at all time points). Subsequently, spots were enumerated using an automated ELISPOT plate reader (AID). A positive ELISPOT response induced by TroVax was reported if the mean spot-forming units per well in response to antigen was ≥3-fold the mean spot-forming units per well in wells containing medium alone and the mean spot-forming units per well in response to antigen is ≥10 and the antigen-specific frequency after immunization was ≥2-fold the frequency before TroVax vaccination. The frequency of antigen-specific cells is reported as 1:*X* PBMCs, where *X* is any value up to 200,000.

Statistical analysis of association between immunologic and clinical responses. Mean 5T4- and MVA-specific ELISA and ELISPOT responses were determined for all evaluable patients from week 2 to 14 or 2 to X + 8. Subsequently, 5T4- or MVA-specific immune responses were correlated with clinical benefit. Two measures of clinical benefit were used: (*a*) change in tumor burden of all target lesions at weeks 14 and X + 8 expressed as a percentage of the baseline tumor burden or (*b*) Response Evaluation Criteria in Solid Tumors (RECIST) response score. The latter was calculated by assigning a score for the tumor responses at weeks 14 and X + 8 such that a score of 1 is progressive disease (PD), 2 is unconfirmed stable disease (SD), 3 is confirmed SD, 4 is unconfirmed partial response (PR), 5 is confirmed PR, 6 is unconfirmed

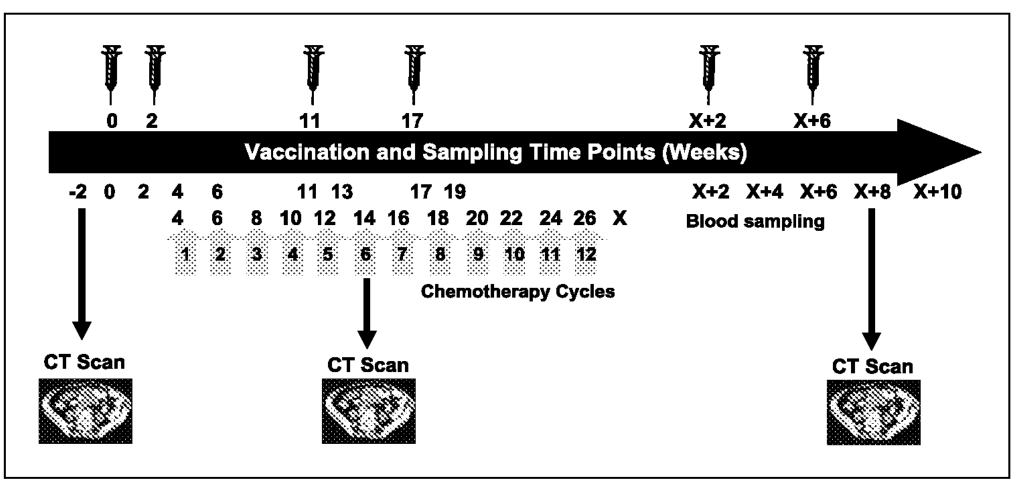

Fig. 1. Vaccination, chemotherapy, and blood sampling schedule. The schematic shows each vaccination time point (*syringe*) and time points at which blood samples were taken for monitoring of immune responses (*below the solid arrow*) and CT scans were done to monitor disease progression.

Table 1. ITT patient characteristics

Patient no.	Age	Sex	Site of target metastatic lesion(s)	No. vaccinations	No. chemotherapy cycles	Evaluable for immune response	Serum CEA (μg/L)*	
							Before	After
101	68	M	Liver, node	6	9	Yes	1	1
102	65	M	Liver	6	9	Yes	12	1
103	65	M	Liver	6	12	Yes	31	2 †
104	59	F	Liver, node	6	12	Yes	56	6
105	56	M	Liver	6	8	Yes	321	5 †
106	54	F	Liver	3	4	No	78	3
107	67	M	Node	6	12	Yes	4	3
108	59	M	Liver	6	12	Yes	1	1
109	66	M	Liver	2	0	No	3,342	9,880
110	55	M	Liver	2	3	No	10	13
111	65	F	Lung	2	3	No	2	3
112	72	F	Liver, lung, node	4	6	No	81	87
113	47	M	Liver	6	12	Yes	21	8
114	56	F	Resection margin, node	6	10	Yes	4	4
115	62	M	Liver, node	1	0	No	54	N/A
116	49	F	Liver	6	12	Yes	635	220
117	51	M	Liver, lung, node	6	12	Yes	1	1

NOTE: The table details the age, sex, main sites of metastatic disease, and the total number of TroVax injections and chemotherapy cycles received. In addition, the level of circulating CEA (μg/L) detectable at baseline is tabulated alongside the lowest level detected at any time point after TroVax vaccination.
Abbreviation: N/A, not available.
*Tabulated measurements represent the lowest levels of circulating CEA detected at either of the two pre-TroVax injection time points and any of the post-TroVax time points.
† Lowest CEA levels occurred following the completion of chemotherapy.

complete response (CR), and 7 is confirmed CR. Unconfirmed responses have been defined as situations whereby the best response (at either time point) has not been confirmed at a later time point (e.g., an unconfirmed PR would be a PR at week 14 followed by PD at week X + 8 or a SD at week 14 followed by a PR at week X + 8). The Spearman correlation was used to investigate potential associations between immune response and clinical benefit. A P value <0.05 was deemed significant.

Results

Patients. In total, 17 patients were enrolled in the trial and included in the intention-to-treat (ITT) population. Six patients withdrew from the trial before becoming evaluable for assessment of immunologic and clinical responses as follows: 2 patients (patients 112 and 109) were withdrawn due to PD, 3 patients (patients 106, 111, and 115) withdrew due to serious adverse events unrelated to TroVax, and 1 patient (patient 110) was lost to follow-up. Only one of the nonevaluable patients (patient 112) was on study for sufficient time to have a protocol-mandated CT scan taken at week 14; therefore, no commentary is made on clinical responses in the ITT group, due simply to the lack of CT scan data. The mean age (±SD) of the ITT population was 59.7 ± 7.2 years (range, 47-72 years). The characteristics of the ITT patient group are detailed in Table 1.

Safety. No TroVax-related serious adverse events were reported in the ITT population. The most frequent adverse event related to TroVax administration was soreness at the site of injection that occurred in seven (41%) patients (Table 2). The other toxicities experienced by patients were in keeping with those expected form oxaliplatin and 5-FU given at these doses.

Antibody responses. 5T4-specific antibody responses were monitored by ELISA at each sampling time point throughout the trial and expressed as a titer (Table 3A). No patient had a detectable 5T4-specific antibody titer before TroVax immunization and one patient (patient 101) failed to show an increase following vaccination. However, 10 patients showed 5T4-specific antibody titers (range, 10-1,280), which were evident after two or more vaccinations in the majority of patients and remained detectable for all, or part, of the period in which patients received chemotherapy. During the period that patients received chemotherapy (week 4 to approximately week 19), the mean titer was 105, which increased moderately to 121 following completion of chemotherapy (weeks X + 2 to X + 14).

MVA-specific antibody responses were also monitored by ELISA at each sampling time point (Table 3B). One patient (patient 102) had a detectable MVA-specific antibody titer (2,000) before TroVax immunization. All patients showed either *de novo* MVA-specific antibody responses or an increase in antibody titer (patient 102) following vaccination with titers ranging from 2,000 to 512,000. Positive responses were detectable after a single vaccination in the majority of patients and remained positive during the period in which patients received chemotherapy. The mean MVA antibody titer for all 11 evaluable patients during the period in which chemotherapy was administered was 22,291, which increased to 46,545 following completion of chemotherapy (weeks X + 2 to X + 14).

TroVax induced IFNγ ELISPOT responses. IFNγ ELISPOT responses to a panel of antigens were monitored using thawed PBMCs directly without any additional *in vitro* restimulation steps. Responses to the positive control CEF peptide pool, 5T4 peptides, and MVA are detailed in Table 4A. Positive responses to the CEF peptide pool were detected in all 11 evaluable

Table 2. Adverse events classified as 'probably' or 'definitely' related to TroVax

	No. events (%)	Grade 1, *n* (%)	Grade 2, *n* (%)	Grade, 3 *n* (%)
Discomfort at injection site	7 (41)	7 (41)		
Rigors/chills	1 (6)	1 (6)		
Myalgia	2 (12)	2 (12)		
Fever/sweating	2 (12)	2 (12)		
Headache	1 (6)		1 (6)	
Hallucinations	1 (6)			1 (6)
Nausea	1 (6)	1 (6)		
Disorientation	1 (6)	1 (6)		
Dizziness	1 (6)	1 (6)		

NOTE: The total numbers of adverse events are listed by toxicity and by toxicity grade.

patients with precursor frequencies ranging from 1:19,230 (0.005%) to 1:600 PBMCs (0.16%). The CEF-specific precursor frequencies were highly consistent throughout the trial monitoring period both before and after TroVax vaccination (data not shown). The mean difference in CEF-specific precursor frequencies detected before TroVax vaccination compared with after vaccination was 1.1 (range, 0.46- to 1.8-fold). Positive ELISPOT responses to MVA were detected in 9 patients at baseline and in all 11 evaluable patients after TroVax immunization. Of the nine patients with preexisting MVA responses, only one (patient 117) showed a >2-fold increase in response following vaccination. Mean MVA-specific precursor frequencies increased from 1:38,800 (range, <1:200,000-1:639) before TroVax vaccination to 1:1,025 (range, 1:1,500-1:589) after vaccination, which constituted a mean 35-fold increase (range, 1- to >203-fold). No patient had a detectable 5T4 peptide-specific IFNγ ELISPOT response before TroVax immunization (frequency, <1:200,000). However, following

Table 3. 5T4- and MVA-specific antibody responses

A. 5T4 antibody responses

Patient no.	5T4-specific antibody titer at time points (wks) after primary immunization													
	-2	0	2	4	6	11	13	17	19	X + 2	X + 4	X + 6	X + 8	X + 10
101	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
102	<10	<10	***10***	***1,280***	***80***	***40***	***40***	***20***	***40***	***20***	***80***	***80***	***80***	***80***
103	<10	<10	<10	***80***	***20***	<10	***20***	***80***	***640***	***80***	***160***	***160***	***320***	***320***
104	<10	<10	<10	<10	<10	<10	***10***	<10	***10***	<10	<10	<10	***40***	***20***
105	<10	<10	<10	***40***	***10***	<10	<10	<10	<10	<10	<10	<10	***160***	***80***
107	<10	<10	<10	***160***	***1,280***	***160***	***640***	***80***	***320***	<10	***160***	***160***	***1,280***	***160***
108	<10	<10	<10	***40***	***40***	<10	***1,280***	***320***	***320***	***20***	***640***	***160***	***640***	***1,280***
113	<10	<10	<10	***160***	<10	<10	***20***	<10	<10	<10	<10	<10	<10	<10
114	<10	<10	<10	***320***	***160***	<10	<10	<10	<10	<10	<10	<10	<10	***<10***
116	<10	<10	***10***	***20***	***20***	<10	<10	<10	***80***	<10	***80***	***40***	***80***	***40***
117	<10	<10	<10	***10***	***10***	<10	<10	<10	***40***	<10	***20***	<10	***160***	***80***

B. MVA antibody responses

Patient no.	MVA-specific antibody titer at time points (wks) after primary immunization													
	-2	0	2	4	6	11	13	17	19	X+2	X+4	X+6	X+8	X+10
101	<2,000	<2,000	<2,000	<2,000	***4,000***	<2,000	<2,000	<2,000	***2,000***	<2,000	***16,000***	***16,000***	***16,000***	***16,000***
102	<2,000	**2,000**	***256,000***	***512,000***	***128,000***	***128,000***	***64,000***	***64,000***	***32,000***	***32,000***	***128,000***	***128,000***	***128,000***	***256,000***
103	<2,000	<2,000	***256,000***	***256,000***	***128,000***	***64,000***	***32,000***	***64,000***	***32,000***	***32,000***	***64,000***	***64,000***	***128,000***	***128,000***
104	<2,000	<2,000	***8,000***	***16,000***	***16,000***	***8,000***	***16,000***	***2,000***	***16,000***	***4,000***	***8,000***	***4,000***	***4,000***	***4,000***
105	<2,000	<2,000	***16,000***	***32,000***	***32,000***	***8,000***	***8,000***	***8,000***	***4,000***	***4,000***	***16,000***	***16,000***	***32,000***	***32,000***
107	<2,000	<2,000	<2,000	***8,000***	***8,000***	***8,000***	***32,000***	***16,000***	***32,000***	***8,000***	***64,000***	***64,000***	***256,000***	***128,000***
108	<2,000	<2,000	<2,000	***16,000***	***8,000***	<2,000	***32,000***	***16,000***	***32,000***	***4,000***	***32,000***	***64,000***	***64,000***	***64,000***
113	<2,000	<2,000	***32,000***	***64,000***	***16,000***	***4,000***	***32,000***	***16,000***	***16,000***	***8,000***	***64,000***	***64,000***	***64,000***	***64,000***
114	<2,000	<2,000	***32,000***	***16,000***	***16,000***	***4,000***	***16,000***	***16,000***	***8,000***	***8,000***	***16,000***	***16,000***	***16,000***	***16,000***
116	<2,000	<2,000	***4,000***	***16,000***	***4,000***	***2,000***	***4,000***	***2,000***	***16,000***	***32,000***	***32,000***	***16,000***	***32,000***	***16,000***
117	<2,000	<2,000	***2,000***	***8,000***	***2,000***	<2,000	<2,000	<2,000	***8,000***	***4,000***	***16,000***	***4,000***	***16,000***	***32,000***

NOTE: Results are expressed as antigen-specific antibody titers [the greatest plasma dilution at which the test sample has a mean absorbance (490 nm) ≥2-fold that of the negative control sample (normal human plasma)] at each sampling time point. Results tabulated in bold text represent positive antibody responses. Results tabulated in bold text and italics represent positive antibody response relative to the preinjection baseline.

vaccination, PBMCs from nine patients responded to 5T4 peptides with precursor frequencies ranging from 1:18,867 to 1:726 PBMCs. If responses to multiple peptide pools (i.e., 5T4-specific polyclonal responses) are analyzed (Table 4B), precursor frequencies in excess of 1:10,000 PBMCs were detected in seven patients, two of whom had precursor frequencies >1:1,000. If the responses to 5T4 protein are added to those detected to 5T4 peptides, nine patients had 5T4-specific polyclonal responses in excess of 1:10,000 and four had responses >1:1,000. The IFNγ ELISPOT responses detected in patient 105 before (weeks -2 and 0) and following TroVax immunization (weeks 13, 19, and X + 2) are shown in Fig. 2.

Table 4. Antigen-specific IFNγ ELISPOT responses

A.

Patient no.	Antigen	Peak antigen-specific T-cell precursor frequency		Fold increase after/before TroVax immunization
		Before TroVax immunization	After TroVax immunization	
101	CEF	1:3,952	1:3,636	1.1
	5T4 Pep #5	≤1:200,000	1:5,988	≥33.4
	MVA	1:1,239	1:737	1.7
102	CEF	1:16,129	1:17,241	0.93
	5T4 Pep #20	≤1:200,000	1:2,380	≥84
	MVA	1:1,034	1:776	1.3
103	CEF	1:10,309	1:19,230	0.5
	5T4 Pep #5	≤1:200,000	1:18,867	≥10.6
	MVA	1:1,263	1:1,163	1.1
104	CEF	1:1,439	1:3,096	0.5
	5T4 Pep #8	≤1:200,000	1:14,705	≥13.6
	MVA	≤1:200,000	1:987	≥202.6
105	CEF	1:1,667	1:943	1.8
	5T4 Pep #13	≤1:200,000	1:1,072	≥186.5
	MVA	1:946	1:1,355	0.7
107	CEF	1:1,754	1:1,941	0.9
	5T4 Pep A1A3B7	≤1:200,000	1:11,111	≥18
	MVA	1:1,172	1:710	1.7
108	CEF	1:640	1:576	1.1
	5T4 Pep #1	≤1:200,000	1:726	≥275
	MVA	1:1,639	1:905	1.8
113	CEF	1:2,325	1:2,857	0.8
	5T4 Pep #12	≤1:200,000	1:2,631	≥76
	MVA	1:1,748	1:1,095	1.6
114	CEF	≤1:10,000	1:6,896	1.4
	5T4 Pep	≤1:200,000	≤1:200,000	1
	MVA	≤1:200,000	1:1,500	≥133
116	CEF	1:2,833	1:2,062	1.4
	5T4 Pep #20	≤1:200,000	1:9,708	≥20.6
	MVA	1:1,047	1:1,456	0.7
117	CEF	1:1,449	1:883	1.6
	5T4 Pep	≤1:200,000	≤1:200,000	1
	MVA	1:16,666	1:589	28.3

B.

Patient no.	Sum peak 5T4 polyclonal precursor frequencies			
	Time point (wk)	Peptides alone	Time point (wk)	Protein + peptides
101	13	1:5,998	X + 8	1:875
102	X + 2	1:2,380	X + 2	1:581
103	X + 2	1:18,867	2	1:9,433
104	19	1:5,494	13	1:3,759
105	19	1:591	19	1:591
107	19	1:11,111	19	1:11,111
108	X + 8	1:726	X + 8	1:449
113	4	1:2,631	4	1:2,631
114	—	<1:200,000	13	1:6,757
116	X+6	1:9,708	X + 6	1:6,289
117	—	<1:200,000	—	<1:200,000

NOTE: A. The peak antigen-specific precursor frequencies detected at baseline (before TroVax immunization) and after TroVax immunization following stimulation of patients' PBMCs with the CEF-positive control peptide pool, 5T4 peptide pools, and MVA. The fold increase in antigen-specific precursor frequency following TroVax immunization is tabulated. B. The peak 5T4-specific responses detected at any time point to ≥1 5T4 peptide pool or 5T4 peptide(s) plus protein (i.e., the sum of individual responses at 1 time point).
Abbreviation: Pep, peptide.

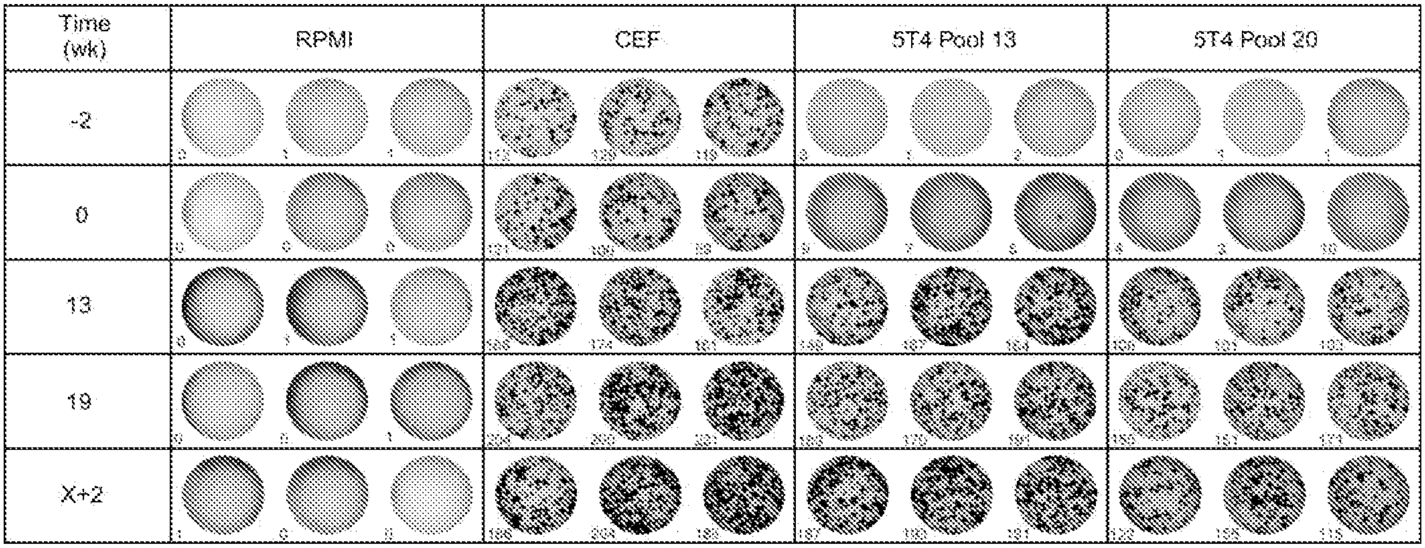

Fig. 2. IFNγ ELISPOT responses detected in patient 105 following incubation of patients' PBMCs with medium alone (RPMI 1640), CEF peptide pool, and two 5T4 peptide pools (pools 13 and 20). Responses from PBMCs taken before TroVax vaccination (weeks -2 and 0) are shown alongside PBMCs recovered during chemotherapy (weeks 13 and 19) and after chemotherapy (week X + 2).

No responses were detected to medium alone (RPMI 1640) and relatively consistent CEF-specific responses were seen (<2-fold difference across all sampling time points). Responses to 5T4 peptide pools 13 and 20 were negative before TroVax vaccination but strongly positive after vaccination, including during the period in which the patient received chemotherapy (weeks 13 and 19).

Clinical responses. The levels of circulating CEA were determined throughout the trial. Table 1 summarizes the levels of circulating CEA present at baseline and the lowest levels detected following TroVax immunization. Of the 17 ITT patients, 11 had elevated CEA levels (>5 μg/L) at baseline and 7 of these showed a >50% reduction at ≥1 time point throughout the trial. In the majority of patients, the nadir in CEA levels occurred during the period in which both chemotherapy and TroVax were administered, although in 2 patients (patients 103 and 105), this occurred following the completion of chemotherapy (i.e., after week X).

Radiological responses to the trial therapy were observed in 5 of 11 (45%) evaluable patients at week 14 (six cycles of chemotherapy and three TroVax administrations; Table 5). As clinical efficacy was not a primary end point in this study, confirmatory CT scans ≥4 weeks later were not mandated. However, CT scans done at week X + 8 (between weeks 28 and 37)

Table 5. Tumor responses detected in all evaluable patients and correlates with immune response

Patient no.	Tumor response		RECIST response score	Tumor burden at X + 8 wk as % of baseline	Mean immune responses (weeks 2-X + 8)			
	Week 14	Week X + 8			MVA ELISPOT	5T4 ELISPOT	MVA antibody	5T4 antibody
101	PR	PR (28 wk)	5	30.8	694.4	31.5	6,000.0	0.0
102	PR	CR (28 wk)	6	0.0	982.7	73.9	145,454.5	160.9
103	SD	PR (37 wk)	4	58.3	577.0	2.0	101,818.2	141.8
104	SD	PR (34 wk)	4	61.4	874.0	3.5	9,272.7	5.5
105	PR	PR (28 wk)	5	48.6	644.0	39.0	16,000.0	19.1
107	SD	SD (34 wk)	3	105.2	935.4	18.1	45,090.9	385.5
108	PR	PR (37 wk)	5	39.5	665.0	39.2	24,363.6	314.5
113	SD	PD (34 wk)	2	95.6	427.0	5.4	34,545.5	16.4
114	SD	PD (33 wk)	2	87.4	337.6	2.9	14,909.1	43.6
116	PR	PD (34 wk)	4	72.9	285.9	3.2	14,000.0	30.0
117	SD	PD (35 wk)	2	102.7	989.3	0.0	5,454.5	21.8
RECIST response score vs immune response			Spearman correlation		0.21	0.77	0.24	0.07
				P	0.54	0.006	0.47	0.83
Change in tumor burden vs immune response			Spearman correlation		-0.06	≥10.64	-0.17	0.08
				P	0.85	0.035	0.61	0.81

NOTE: Tumor responses reported at weeks 14 and X + 8 (the actual week after primary immunization is indicated in parentheses) are tabulated alongside the RECIST response score and the total tumor burden at week X + 8 expressed as a percentage of the tumor burden at baseline. In addition, the magnitude of mean 5T4- or MVA-specific ELISA and ELISPOT responses (between weeks 2 and X + 8) are tabulated alongside the RECIST response score or change in tumor burden at week X + 8 expressed as a percentage of baseline. The Spearman correlation and *P* value are noted.

showed continued PRs in three patients and a CR in one patient (patient 102). Table 5 also summarizes that for all but two patients, the response observed at week 14 was maintained. The RECIST response score and the total tumor burden at week X + 8 expressed as a percentage of the total tumor burden at baseline. The median survival was 68 weeks in the 17 ITT patients and 118 weeks in the 11 evaluable patients.

Correlation of clinical and immunologic responses. Mean 5T4- and MVA-specific antibody and ELISPOT responses detected between weeks 2 to 14 (i.e., mean responses across 5 sampling time points) and weeks 2 to X + 8 (i.e., mean responses across 11 sampling time points) were calculated and compared with indicators of clinical benefit (RECIST response score or change in tumor burden; Table 5). No association was observed between 5T4- or MVA-specific antibody responses and clinical benefit. However, significant correlations were detected between 5T4-specific ELISPOT responses and RECIST response score (P = 0.006) and change in tumor burden at week X + 8 (P = 0.035). No significant correlations were detected with MVA-specific ELISPOT responses.

Discussion

Currently, few clinical studies have investigated the use of a cancer vaccine in combination with chemotherapy due to the perceived negative effect of cytotoxic agents on cells of the immune system. In addition to direct effects on the immune system, most chemotherapies are thought to kill tumor cells through the induction of apoptosis, which has traditionally been associated with the induction of tolerance rather than immunity (16). However, numerous pathways have been postulated by which some chemotherapy regimens could augment immunotherapy; these include enhanced cross-presentation of antigens, partial activation of dendritic cells, and promotion of long-term antigen-independent memory (reviewed in ref. 13). For example, the use of low-dose cyclophosphamide has been shown to enhance immune responses against several tumor antigens (17). Such immunostimulatory properties were linked to the ability of low-dose cyclophosphamide to decrease both the number and the activity of regulatory T cells (18, 19). Indeed, decreases in the percentage of regulatory T cells have been associated with a switch from tumor escape to tumor rejection (18, 20).

We have shown that the administration of TroVax in combination with 5-FU/folinic acid and oxaliplatin is both safe and capable of inducing potent 5T4-specific immune responses. Of the 11 patients who completed the TroVax vaccination schedule, all mounted 5T4-specific cellular and/or humoral immune responses. The immune responses detected to 5T4 were, in general, of greater magnitude and longevity than those detected in a phase I/II study in which TroVax was administered as a monotherapy to stage IV colorectal cancer patients (12). Indeed, both the frequency and the magnitude of 5T4-specific immune responses are, to our knowledge, some of the highest reported tumor-associated antigen–specific responses induced in cancer patients following vaccination (21). Antigen-specific precursor frequencies in excess of 1 per 1,000 PBMCs are usually only detected when responses to viral antigens are analyzed. Indeed, the responses to 5T4 reported in this study were frequently as high as those detected to the MVA viral vector. This was true even in circumstances in which a preexisting response to MVA was detected or following the induction of a potent antibody response that frequently occurred following a single TroVax vaccination.

Given that this trial was a small, open-label single-arm study, in which tumor response was not a primary end point, it is not appropriate to comment extensively on the clinical responses detected in this patient cohort. However, the following observations can be made: the frequency of observed CR/PR were of a similar order to those seen in pivotal studies using similar chemotherapy regimens alone (22, 23), indicating no negative effect of TroVax on delivery or activity of the chemotherapy. Although no firm conclusions can be drawn from this study about possible synergism between TroVax and chemotherapy, it was encouraging that a significant correlation between 5T4-specific immune response and clinical benefit was detected. It could be argued that the ability to mount a 5T4-specific immune response is a function of the immunocompetence and general health of the patient, which could explain the trend with clinical benefit. However, immune responses to MVA represent a good internal control for immunocompetence and no significant correlates existed between the magnitude of MVA responses and clinical benefit.

Despite recent progress, a pressing requirement for improved therapies to combat advanced cancer remains. Combining different treatment modalities, such as biological and cytotoxic agents, has scientific and clinical rationale as long as the combined toxicities are not excessive. Here, we have shown that the immunologic efficacy of TroVax has been maintained in the context of a standard of care chemotherapy regimen without additional toxicity.

These data provide further support that combining a cancer vaccine, such as TroVax, with chemotherapy may be beneficial. Further studies aimed at characterizing the timing of vaccination relative to chemotherapy and identifying the optimum chemotherapy regimen may lead to increased clinical benefit. In conclusion, we have shown that TroVax is safe and highly immunogenic when administered to patients alongside 5-fluorouracil/folinic acid and oxaliplatin. Furthermore, a significant correlation between 5T4-specific immune responses and clinical benefit was detected. We believe that these observations provide good justification for the continued development of TroVax alongside other standard of care therapies.

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